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Exposure to Total and Protein-Unbound Rifampin Is Not Affected by Malnutrition in Indonesian Tuberculosis Patients

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Nutritional status may have a profound impact on the pharmacokinetics of drugs, yet only few data are available for tuberculosis (TB) drugs. As malnutrition occurs frequently among TB patients, we assessed the effect of malnutrition on the steady-state pharmacokinetics of total and protein-unbound rifampin during the intensive phase of TB treatment. In a descriptive pharmacokinetic study in Bandung, Indonesia, patients received a fixed standard rifampin dose of 450 mg once daily during the intensive phase of TB treatment. A full pharmacokinetic curve for rifampin was recorded, and total and unbound concentrations of rifampin were analyzed in all samples. Rifampin pharmacokinetic parameters were compared between severely malnourished (BMI of <16.0 kg/m^2), malnourished (BMI of <18.5 kg/m^2), and well-nourished (BMI of ≥18.5 kg/m^2) individuals. No difference in total and protein-unbound pharmacokinetic parameters between severely malnourished ($n = 7$), malnourished ($n = 11$), and well-nourished ($n = 25$) patients could be demonstrated. In addition, no significant correlation between BMI and exposure (area under the concentration-time curve from 0 to 24 h [AUC_{0-24}] and maximum concentration of drug in serum [C_{max}]) was found. Females had significantly higher total AUC_{0-24} (geometric mean, 59.2 versus 48.2 h · mg/liter; $P = 0.02$) and higher unbound AUC_{0-24} (geometric mean, 6.2 versus 4.8 h · mg/liter; $P = 0.02$) than males. Overall, a marked 2-fold interindividual variation in the free fraction was observed (7.6 to 15.0%; $n = 36$). Nutritional status and BMI do not appear to have a major effect on total and protein-unbound pharmacokinetic parameters of rifampin in Indonesian subjects. The large interindividual variability in the free fraction of rifampin suggests that protein-unbound rather than total rifampin concentrations should preferably be used to study exposure-response relationships.

Inadequate exposure to rifampin and other antituberculosis (anti-TB) drugs may contribute to a suboptimal clinical response in anti-TB treatment. This follows from a recent study performed in a preclinical model, showing that pharmacokinetic variability is an important factor in the emergence of multidrug-resistant TB (1). Furthermore, a meta-analysis of clinical studies showed that pharmacokinetic variability for a single drug (isoniazid) in multidrug TB regimens is associated with therapy failure and acquired drug resistance (2). A number of clinical studies have also reported associations between low concentrations of anti-TB drugs and poor treatment response (3–8), but this association was not found in other studies (9, 10), including one of our studies on plasma rifampin concentrations in Indonesian TB patients (11).

For rifampin and other TB drugs, pharmacokinetic variability and low exposure may be affected by various factors, including gender, comorbidity (HIV/AIDS or diabetes mellitus), genetics, drug formulation, and malnutrition (3, 12–16). Malnutrition occurs frequently among TB patients. A case ($n = 121$)-control ($n = 371$) study in Indonesia documented malnutrition in 87% and 33% of cases and controls, respectively (17). A bidirectional interaction exists between malnutrition and TB (18, 19). On the one hand, malnutrition impairs immune function and increases the susceptibility to development of active TB. At the same time, TB leads to severe abnormalities in protein metabolism and loss of lean tissues and fat reserves. It is known that nutritional status can have a profound impact on the pharmacokinetics of drugs (20, 21), yet few data are available for TB drugs, and we are aware of only one publication on the effect of malnutrition on the exposure to rifampin (12).

In pharmacokinetic studies, measurement of rifampin concentrations in plasma or serum usually relates to the total (protein-unbound plus protein-bound) concentration of a drug. An equilibrium between total and protein-unbound concentrations is commonly assumed, yet free rather than total drug concentrations are preferably used in concentration-response evaluations (22), as only protein-unbound drugs are pharmacologically active and diffuse or are being actively transported into tissues and to the sites of action (23, 24). In a previous study among Indonesian TB patients (11), we confined measurements to total concentrations of rifampin, and this may be one of several possible explanations for the absence of a concentration-response relationship in that study. Importantly, malnutrition and associated low concentra-

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tions of drug-binding plasma proteins may cause a change in the equilibrium between protein-unbound and -bound concentrations, which renders the total drug concentrations misleading (24–26). This means that both total and protein-unbound plasma concentrations should be evaluated when studying the effects of malnutrition on the pharmacokinetics of a drug.

The primary objective of this study was to assess the effect of malnutrition on the steady-state pharmacokinetics of total and protein-unbound rifampin during the intensive phase of TB treatment in Indonesian TB patients. As a secondary objective, we evaluated the interindividual variability in exposure to protein-unbound rifampin, as we feel this may provide relevant information to understand exposure-response relationships for this pivotal TB drug.

MATERIALS AND METHODS

Subjects. Study subjects were Indonesian patients with pulmonary TB in the intensive phase of treatment. Diagnosis of pulmonary TB was based on clinical symptoms and chest X-ray examination, confirmed by microscopic detection of acid-fast bacilli. Patients were excluded if they had a body weight (BW) above 55 kg, were below 18 or above 55 years of age, were pregnant or lactating, used any type of comedication that may influence the pharmacokinetics of TB drugs, or had liver or kidney abnormalities (including abnormal liver or renal function parameters) or any known history or medical condition that might affect the pharmacokinetics of TB drugs, such as diabetes mellitus, HIV infection, diarrhea, or vomiting.

Study design. This was a descriptive pharmacokinetic study conducted in an urban outpatient tuberculosis clinic in Bandung, Indonesia. Patients were prospectively and consecutively recruited from the control arm of an intervention study on nutritional supplementation in TB patients. Subsequently, the cohort was completed with data from patients who participated in a clinical trial on high-dose rifampin and who fulfilled the inclusion and exclusion criteria (27). During the intensive phase of TB treatment, all eligible patients received a fixed standard rifampin dose of 450 mg once daily, roughly corresponding to 10 mg/kg in Indonesian people (for people below 55 kg), combined with once-daily isoniazid (300 mg), pyrazinamide (1,500 mg), and ethambutol (750 mg). All patients received TB drugs from the same manufacturer (PT Kimia Farma, Bandung, Indonesia), formulated in separate tablets. The bioequivalence of the rifampin tablets and an international reference standard has been established before (28).

A full pharmacokinetic curve for rifampin was recorded between 2 and 6 weeks after the start of TB treatment, when steady state for the TB drugs can be expected (29). Body weight and height (to calculate body mass index [BMI]) and concomitant drug use were also assessed at the pharmacokinetic sampling day. In addition, plasma protein albumin was measured, considering that 30 to 41% of protein-bound rifampin is associated with the serum albumin fraction (30). Informed consent was obtained from all subjects, and the study was approved by the Independent Ethics Committee, Faculty of Medicine, University of Padjadjaran, Bandung, Indonesia.

Blood sampling, bioanalysis, and pharmacokinetic data analysis. Patients refrained from the intake of any food or any drugs (other than study medication) starting from 11:00 p.m. on the day preceding the pharmacokinetic assessment until 4 h after the intake of study medication. TB drugs were taken with 230 ml of still water. Serial blood samples (10 ml) were collected from the antecubital vein just before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after observed TB drug intake. Plasma was immediately separated, frozen at −20°C, and transferred to −80°C within 72 h until transport on dry ice to The Netherlands for bioanalysis.

Bioanalysis. The total (protein-bound plus -unbound) plasma concentrations of rifampin were determined with a validated high-performance liquid chromatographic (HPLC) method with UV detection as described previously (27). The lower limit of quantitation for this method was 0.28 mg/liter.

In addition to total plasma concentrations, protein-unbound concentrations of rifampin were measured in all obtained samples. Measurements were based on ultrafiltration to separate bound from unbound rifampin, followed by HPLC. Briefly, 0.5 ml of plasma was added into a Centrifree YM-30 tube (Millipore, Amsterdam, The Netherlands). Plasma was centrifuged for 15 min at 1,650 × *g* at 25°C with a Rotanta 46 R, rotor 4445 (radius 164, <45°). The clear ultrafiltrate was placed in a thermostated autosampler (4°C), and 50 μl of this solution was injected in the HPLC system. The analytical column was an OmniSpher 5 C_{18} column (250 by 4.6 mm [inner diameter]; particle size, 5 μm) protected by a Chromguard RP ss 10- by 3-mm column (Varian, Middelburg, The Netherlands). The mobile-phase components were 30% acetonitrile and 70% 10 mM phosphate buffer (pH 5), run during a HPLC gradient with different flow rates. The total run time was 17 min. UV detection was set at 334 nm. The average accuracy of ultrafiltrate spiked with rifampin was 107%. Intraday imprecision in measurement of rifampin in ultrafiltrate varied from 1.4 to 2.4%, interday imprecision varied from 0% (i.e., there was no additional variation upon intraday imprecision as a result of performing the assay on different days) to 3.6%, and overall precision varied from 1.4 to 3.9%, depending on the concentration measured. The range of the method for unbound rifampin plasma concentrations was from 0.06 mg/liter (limit of quantitation) to 13 mg/liter.

Pharmacokinetic analysis. Pharmacokinetic parameters were assessed using standard noncompartmental methods in WinNonLin version 5.3 (Pharsight Corporation) as described before (27). Unbound fractions were calculated by dividing the unbound area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) by the total AUC_{0-24} for all study subjects.

Statistical analysis. Based on available data for the pharmacokinetics of rifampin in Indonesian patients (27), it was calculated that a minimum of 15 patients per group were required to detect a difference of 25% in the AUC_{0-24} of total rifampin with a significance level of 0.05 and a power of 80% (two-sided test).

Patients were first divided into three subgroups based on criteria for nutritional status as proposed by the World Health Organization (31). Patients with a BMI of <16.0 kg/m^2 were considered severely malnourished, patients with a BMI of <18.5 kg/m^2 were regarded as malnourished, and patients with a BMI of ≥18.5 kg/m^2 were considered to have no malnutrition. Differences in pharmacokinetic parameters between malnourished patients as well as the severely malnourished subgroup versus well-nourished patients were assessed with independent-sample *t* tests on logarithmically transformed pharmacokinetic parameters. Time to maximum concentration of drug in serum (T_{max}) values were not transformed and were compared using the Mann-Whitney U test. Apart from categorizing patients in groups with predefined BMI values, BMI was also evaluated as a continuous variable. AUC_{0-24} and maximum concentration of drug in serum (C_{max}) values for both total and unbound rifampin plasma concentrations were correlated with BMI using Spearman's rho on the untransformed pharmacokinetic parameters.

To evaluate the confounding effect of other possible determinants of exposure to protein-unbound and total rifampin, similar univariate analyses were performed to assess the effects of gender, weight, age, and plasma albumin concentration on the log-transformed AUC_{0-24} and C_{max} values for the total and unbound concentrations of rifampin. After the univariate analyses, a multiple linear regression analysis was performed to assess the variation in log-transformed AUC_{0-24} and C_{max} attributable to the presence of those variables that emerged from the univariate analyses.

To assess the interindividual variability in pharmacokinetics as a secondary objective, all patients were combined in one group. First, the central tendency and spread in each pharmacokinetic parameter (protein-unbound and total rifampin) were described with a geometric mean, geometric coefficient of variation (GCV) (standard deviation [SD] of ln-

TABLE 1 Demographic, clinical, and laboratory characteristics of the study population

Characteristic	Value ($n = 36$)[a]
Age (yr)	35 (18–55)
Male sex (%)	39
Wt (kg)	44 (34–53)
BMI (kg/m^2)	19.0 (13.5–22.6)
Severely malnourished (BMI, <16 kg/m^2; $n = 7$)	15.3 (13.5–15.8)
Malnourished (BMI, 16–18.49 kg/m^2; $n = 4$)	16.3 (16.1–16.4)
Normal BMI (BMI, ≥18.5 kg/m^2; $n = 25$)	19.6 (18.7–22.6)
Albumin (g/dl)	3.8 (2.6–4.2)
Hemoglobin (g/dl)	11.7 (8.9–14.4)
Rifampin dose (mg/kg)	10.3 (8.5–13.2)
Severely malnourished (BMI, <16 kg/m^2; $n = 7$)	12.4 (10.5–13.2)
Malnourished (BMI, 16–18.49 kg/m^2; $n = 4$)	11.4 (10.5–13.2)
Normal BMI (BMI, ≥18.5 kg/m^2; $n = 25$)	9.6 (8.5–11.5)

[a] Data are presented as median (minimum–maximum) unless stated otherwise.

transformed data × 100%), and range, apart from T_{max}, which was presented as median and range. The variability in average (arithmetic mean) percent unbound rifampin (unbound AUC_{0-24}/total AUC_{0-24}) was described with a coefficient of variation (CV) (SD/mean × 100%) determined over all individual pharmacokinetic curves. Second, the geometric mean total and protein-unbound plasma concentrations and average ratios of unbound to total rifampin were calculated for all individual samples at each sampling time point.

All statistical evaluations were performed with SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL). *P* values of less than 0.05 were considered statistically significant in all analyses.

RESULTS

Patients. Thirty-six patients with pulmonary TB were included in the study. Characteristics of the patients are presented in Table 1. The majority of the patients were female (61%). Twenty-five patients had a normal BMI (BMI of ≥18.5 kg/m^2), and 11 patients were malnourished (BMI of <18.5 kg/m^2), of which seven patients were severely malnourished (BMI of <16.0 kg/m^2). As all patients received a fixed dose of 450 mg of rifampin; the dosage of rifampin per kilogram of body weight was somewhat higher among malnourished patients than among those with a normal BMI (11.6 mg/kg BW versus 9.7 mg/kg BW [geometric mean]). Albumin concentrations were within the normal range for severely malnourished, malnourished, and well-nourished patients: 3.3, 3.4, and 3.8 g/dl (geometric mean), respectively. Albumin concentrations in severely malnourished and malnourished patients did not differ significantly from those in well-nourished patients ($P = 0.12$ and $P = 0.19$, respectively).

Effect of malnutrition on the pharmacokinetics of rifampin. The geometric mean AUC_{0-24} of rifampin did not differ between patients with malnutrition (BMI of <18.5 kg/m^2) and patients with a normal BMI, for both the total AUC_{0-24} (54.8 versus 54.6 h · mg/liter; $P = 0.96$) and the unbound plasma AUC_{0-24} (5.7 versus 5.6 h · mg/liter; $P = 0.95$) (Table 2). Total and unbound geometric mean C_{max} and other rifampin pharmacokinetic parameters, especially the primary parameters clearance and volume of distribution, also were similar in the two nutrition groups (Table 2). Severely malnourished patients (BMI of <16.0 kg/m^2) showed no differences in AUC_{0-24} compared to patients with a normal BMI either; geometric mean values for total and protein-unbound AUC_{0-24} were 56.7 and 6.1 h · mg/liter, respectively, among severely malnourished patients, compared to 54.6 and 5.6 h · mg/liter, respectively, for patients with a normal BMI ($P = 0.73$ and 0.53, respectively). Evaluation of C_{max} values yielded similar results; geometric mean values for total and protein-unbound C_{max} were 10.7 and 1.0 mg/liter, respectively, among severely malnourished patients, compared to 10.9 and 1.1 mg/liter, respectively, in patients with a normal BMI ($P = 0.91$ and $P = 0.65$, respectively).

TABLE 2 Steady-state pharmacokinetics of total and unbound rifampin based on full pharmacokinetic curves of the total cohort

Parameter in plasma[a]	Value for group[b]			*P* value
	Total ($n = 36$)	BMI < 18.5 ($n = 11$)	BMI ≥ 18.5 ($n = 25$)	
Total rifampin				
AUC_{0-24} (h · mg/liter)	54.7 (32.6–88.8)	54.8 (32.6–85.0)	54.6 (35.0–88.8)	0.96[d]
C_{max} (mg/liter)	10.9 (6.4–16.6)	10.9 (6.4–16.6)	10.9 (7.1–16.3)	0.95[d]
T_{max} (h) (median)	2.0 (1.0–4.0)	2.5 (1.0–4.0)	1.5 (1.0–4.0)	0.17[e]
CL/*F* (liters/h)	8.2 (5.1–13.8)	8.2 (5.3–13.8)	8.2 (5.1–12.9)	0.96[d]
V/*F* (liters)	24.1 (15.1–57.4)	25.1 (16.6–36.0)	23.7 (15.1–57.4)	0.63[d]
Half-life (h)	2.0 (1.4–3.8)	2.1 (1.4–2.8)	2.0 (1.5–3.8)	0.47[d]
Unbound rifampin				
AUC_{0-24} (h · mg/liter)	5.6 (2.9–9.8)	5.7 (2.9–8.7)	5.6 (3.2–9.8)	0.95[d]
C_{max} (mg/liter)	1.1 (0.7–2.2)	1.0 (0.7–2.0)	1.1 (0.7–2.2)	0.45[e]
T_{max} (h) (median)	2.0 (1.0–4.0)	2.5 (1.0–4.0)	1.5 (1.0–4.0)	0.06[d]
CL/*F* (liters/h)	79.8 (45.9–156)	79.3 (51.9–156)	80.0 (45.9–142)	0.95[d]
V/*F* (liters)	320 (107–741)	337 (161–654)	313 (108–747)	0.65[d]
Half-life (h)	2.8 (1.2–6.8)	2.9 (2.0–4.0)	2.7 (1.2–6.8)	0.52[d]
Unbound fraction				
AUC ratio (%) (avg)[c]	10.5 (7.6–15.0)	10.5 (8.9–14.9)	10.5 (7.6–15.0)	0.99[d]

[a] CL, clearance; V, volume of distribution.
[b] Data are presented as geometric mean (minimum–maximum) unless stated otherwise.
[c] The AUC ratio was calculated by dividing unbound AUC_{0-24} by total AUC_{0-24} in all subjects.
[d] By independent-sample *t* test on log-transformed pharmacokinetic parameters between malnourished and well-nourished patients.
[e] By Mann-Whitney U test between malnourished and well-nourished patients.

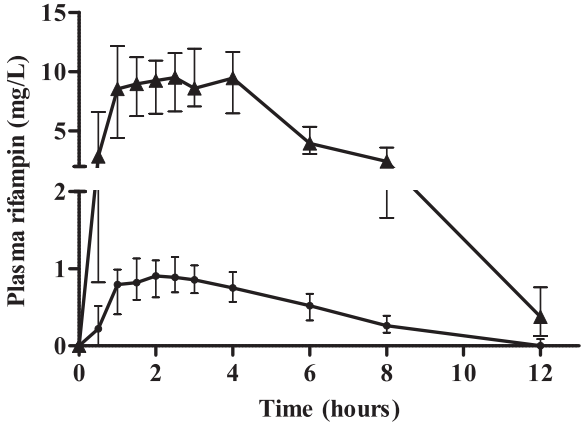

FIG 1 Total (▲) and protein-unbound (●) concentrations of rifampin (mg/liter) at various sampling time points postdose. Concentrations are presented as median ± interquartile range (IQR).

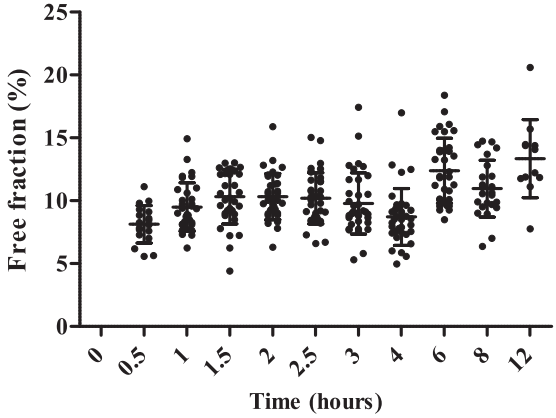

FIG 2 Percent protein-unbound rifampin (free fraction) at various sampling time points postdose. Bars represent arithmetic mean ± SD.

Since patients who were severely malnourished or malnourished had a lower body weight while receiving the same fixed 450-mg dose of rifampin, a similar AUC_{0-24} is explained by a trend toward a higher clearance per kilogram in both severely malnourished [0.21 liter/(h · kg)] and malnourished [0.21 liter/(h · kg)] patients versus well-nourished patients [0.18 liter/(h · kg)] ($P = 0.09$ and $P = 0.07$, respectively).

Apart from categorizing patients in groups with predefined BMI values, BMI was also evaluated as a continuous variable. There was no significant correlation between BMI and AUC_{0-24} for both total and unbound rifampin plasma concentrations (Spearman's rho, 0.035 and −0.055, respectively; $P = 0.84$ and $P = 0.75$, respectively), nor was there a significant correlation between BMI and C_{max} for total and unbound rifampin plasma levels (Spearman's rho, 0.000 and 0.095, respectively; $P = 1.0$ and $P = 0.58$, respectively).

Effects of other determinants on the pharmacokinetics of rifampin. In univariate analyses, gender emerged as the only significant determinant of total and unbound plasma rifampin pharmacokinetic parameters. Females were found to be exposed to higher concentrations of rifampin than males, as indicated by a significantly higher total AUC_{0-24} (geometric mean, 59.2 versus 48.2 h · mg/liter; $P = 0.02$) and higher unbound AUC_{0-24} (geometric mean, 6.2 versus 4.8 h · mg/liter; $P = 0.02$). Total and unbound C_{max} values did not differ significantly between the two groups ($P = 0.16$ and $P = 0.22$, respectively), as was also the case for T_{max} ($P = 0.23$ and $P = 0.22$, respectively). Univariate analyses did not show significant correlations for the determinants body weight and age (data not shown).

As females were overrepresented in the group of patients with a normal BMI (68%) compared to patients with a BMI below 18.5 kg/m^2 (45%) ($P = 0.20$), multiple linear regression analyses were performed to disentangle the effects of gender and BMI. These analyses showed that gender was a significant predictor of the AUC_{0-24} of total and protein-unbound rifampin ($P = 0.02$ and $P = 0.01$, respectively), yet BMI was not ($P = 0.52$ and $P = 0.35$, respectively). Gender alone explained 15% of the variation (r^2) in both the total and protein-unbound AUC_{0-24}.

Variability in total and protein-unbound rifampin concentrations. To assess the interindividual variability in pharmacokinetics, all patients were combined in one group. Both total and protein-unbound rifampin concentrations showed considerable variation. The geometric mean total AUC_{0-24} for all 36 subjects was 54.7 h · mg/liter, with a GCV of 26% and a 2.4-fold interindividual variation in total AUC_{0-24}, ranging from 32.6 to 88.8 h · mg/liter (Table 2 and Fig. 1). The geometric mean unbound AUC_{0-24} amounted to 5.6 h · mg/liter, with a GCV of 32% and a 3.4-fold interindividual variation in unbound AUC_{0-24}, ranging from 2.9 to 9.8 h · mg/liter (Table 2 and Fig. 1). Therefore, interpatient variabilities in AUC_{0-24} for total and unbound rifampin were considered to be comparable. The same applied for C_{max}, with GCVs of 27% and 30% for total and protein-unbound rifampin, respectively. Overall, a significant correlation was found between total and unbound plasma rifampin AUC_{0-24} (Spearman's rho, 0.816; $P < 0.001$) and between C_{max} values (Spearman's rho, 0.696; $P < 0.001$). The arithmetic mean percent unbound AUC_{0-24} was found to be 10.5%, with a CV of 18% and a marked 2-fold interindividual variation in percent unbound rifampin, ranging from 7.6 to 15.0%.

Figure 2 shows the percent protein-unbound rifampin (free fraction) at the various sampling time points, and Table 3 summarizes the mean total and unbound rifampin concentrations, unbound fractions, and interindividual variabilities at the various sampling time points. The free fraction seemed to be slightly higher in samples taken later in the pharmacokinetic curve, with lower total concentrations (Fig. 2 and Table 3).

DISCUSSION

To our knowledge, the present study is the first to obtain both total and protein-unbound full pharmacokinetic curves of rifampin during the intensive phase of TB treatment in malnourished and well-nourished TB patients. The study shows comparable geometric mean total and protein-unbound AUC_{0-24} and C_{max} values in patients who are severely malnourished (BMI of <16.0 kg/m^2), malnourished (BMI of <18.5 kg/m^2), and well nourished (BMI of ≥18.5 kg/m^2). In addition, there was no significant correlation between BMI and AUC_{0-24} or C_{max} for both total and unbound rifampin plasma levels.

The absence of an effect of malnutrition on rifampin exposure in this study is in contrast with the results by Polasa et al. (12). They found that the AUC of a single dose of rifampin was 29% lower in undernourished (BMI of <18 kg/m^2) than in well-nour-

TABLE 3 Mean total and unbound rifampin concentrations, unbound fraction, and interindividual variability at each sampling time point

Time postdose (h)	Concn (mg/liter)[a]		Protein-unbound fraction (%)[b]	n[c]
	Protein unbound	Total plasma		
0.5	0.3 (67; 0.1–0.8)	2.7 (103; 0.3–9.1)	8.1 (18; 5.6–11.1)	18
1	0.7 (71; 0.08–2.2)	6.5 (102; 0.1–16.6)	9.5 (20; 6.2–14.9)	33
1.5	0.8 (71; 0.08–1.7)	6.8 (93; 0.2–15.8)	10.3 (21; 4.4–13.0)	34
2	0.8 (62; 0.08–1.7)	7.3 (71; 0.8–15.0)	10.3 (18; 6.3–15.6)	35
2.5	0.9 (46; 0.14–1.8)	8.5 (40; 2.1–15.5)	10.2 (20; 6.6–15.0)	36
3	0.8 (31; 0.5–1.6)	8.9 (30; 5.1–15.1)	9.8 (25; 5.3–17.4)	36
4	0.7 (38; 0.4–1.7)	8.7 (33; 4.2–15.0)	8.7 (26; 5.0–17.0)	36
6	0.5 (41; 0.2–1.0)	3.9 (33; 2.1–7.3)	12.4 (21; 8.5–18.4)	36
8	0.3 (48; 0.1–0.6)	2.4 (47; 1.0–6.0)	11.0 (21; 6.4–14.7)	24
12	0.1 (43; 0.06–0.2)	0.5 (68; 0.1–1.5)	13.3 (23; 7.8–20.6)	12

[a] Values are geometric means (GCV; range); includes concentrations greater than the limit of quantitation.
[b] Values are arithmetic means (CV; range); includes concentrations greater than the limit of quantitation.
[c] Number of patients for whom a protein-unbound fraction could be calculated based on quantifiable unbound and total rifampin concentrations.

ished healthy volunteers, with plasma peak concentrations in undernourished persons being 50% of that observed in well-nourished individuals. In the study by Polasa et al., plasma protein binding was determined with equilibrium dialysis in peak samples only, and a higher unbound fraction was found in undernourished volunteers. These data suggested that the decrease in total exposure was at least partly compensated by an increase in the free fraction. Polasa et al. also showed apparently lower rifampin AUC and C_{max} values in actual undernourished TB patients who had used rifampin for 4 to 6 months, yet the comparison of steady-state AUC and C_{max} with the single-dose data in healthy volunteers is not valid, considering that rifampin decreases its own exposure upon repeated administration (autoinduction) (30). Nevertheless, average plasma protein binding for rifampin was low in these patients as well (42.99%) (12), suggesting a shift in the equilibrium between total and protein-unbound concentrations.

Indeed we had anticipated that hypoalbuminemia (or a decrease in other proteins relevant to binding of drugs) might result in changes in the total rifampin AUC but *not* in the protein-unbound free AUC in malnourished TB patients, as we considered that changes in plasma protein binding due to protein deficiency are very similar to the situation occurring with drug displacement interactions (24, 25). In this situation, measuring the total concentrations would be misrepresentative for the free, pharmacological active concentrations, yet in our study, there was even no trend for a decrease in exposure to total or protein-unbound rifampin in malnourished or severely malnourished versus well-nourished TB patients. Furthermore, our study did not show a correlation between BMI and the steady-state protein-unbound and total rifampin exposure in our cohort. An explanation for our findings might be that malnourished patients in our study had higher mean albumin levels than the individuals in the study by Polasa et al.: 3.8 g/dl ($\geq$18.5 kg/m^2), 3.4 g/dl ($<$18.5 kg/m^2), and 3.3 g/dl ($<$16.0 kg/m^2) versus 3.6 ($\geq$18.0 kg/m^2) and 2.5 g/dl ($<$18.0 kg/m^2). This indicates that the nutritional status of patients in our study may be better, suggesting that any effect of malnutrition mediated through altered binding to albumin was less likely to occur in our patient population. In contrast, it can also be argued that serum albumin is a suboptimal indicator of nutritional status, especially in marasmic populations, as albumin synthesis can be maintained in such states (32, 33).

Whereas malnutrition did not affect exposure to rifampin, females showed significantly higher total and unbound AUC_{0-24}, also when corrected for BMI. In studies with predominantly Caucasian patients, higher serum rifampin concentrations were found in females, and this could not be explained by differences in body weight (30, 34). In Indonesian patients, two of our previous studies found a relationship between gender and rifampin pharmacokinetics (35, 36), but another study did not (11). In African subjects, female patients also showed higher exposures to rifampin (15). An explanation may be that females generally have a lower lean body mass than males. Thus, for drugs that are differentially distributed between water and fat, different drug concentrations can be found in subjects with the same weight (37). However, it should be noted here that gender explained only 15% of the variation (r^2) in both the total and protein-unbound AUC_{0-24}. Thus, other determinants are probably more important in causing the (high) variation in rifampin exposures.

With respect to interindividual variability, a marked 2-fold interindividual variation in the unbound rifampin fraction (unbound AUC_{0-24}/total AUC_{0-24}), between 7.6 and 15.0%, was observed. In addition, the variation in the free fraction at the various sampling time points was found to be considerably high (Fig. 2), with CVs ranging from 18 to 26% (Table 3). This large interindividual variability in the free fraction shows that measurement of solely total concentrations could be misrepresentative of the actual exposure. This suggests that protein-unbound rifampin concentrations should be considered to evaluate along with total plasma concentrations for assessment of exposure-response relationships in clinical studies. The same may apply to individualization of rifampin dosing based on plasma concentration measurements (therapeutic drug monitoring) in those settings where this technique can be used in patient care. In light of this observed high interindividual variation, the slight increase in the average unbound fraction of rifampin found in the lower total concentrations later in the pharmacokinetic curve (Fig. 2) might be less relevant, also when considering the relatively small number of patients included in this study. In addition, we cannot exclude that this small effect is an artifact related to ultrafiltration as a means to measure protein binding.

In the current study, we measured free rifampin concentrations using ultrafiltration performed at 25°C, as we also do for other drugs in routine patient care. In ultrafiltration, centrifugal forces are employed as the driving force for the passage of plasma water across a filter membrane (38). Besides ultrafiltration, other methodologies are available to determine plasma protein binding of drugs, such as ultracentrifugation and equilibrium dialysis. For rifampin all these techniques have been used. The review by Kenny and Strates states that in case of equilibrium dialysis, various rifampin binding values have been reported based on variable incubation temperatures, protein concentrations, and dialysis times (30). However, the specific effect of temperature on the rifampin-protein complex was not mentioned there or in the literature. For other drugs, an increase in experimental ultrafiltration temperatures has been associated with an increase in the free fraction (39–41), but as far as we know this has not been reported for rifampin. Indeed, we found that measurement of free rifampin at 37°C resulted in a small increase in the rifampin-free fraction

(mean change in free fraction, +1.2%; median change, +1.1%; range, −0.7 to +3.5%, $n = 10$ rifampin concentrations measured, $P = 0.029$ [paired t test]) (unpublished data). This effect of temperature on the rifampin free fraction is small and is unlikely to have affected the findings in our study.

This study has its limitations. First, the number of individuals included in the malnourished group seems somewhat low with 11 individuals, as a number of 15 individuals per group was calculated to be required to detect a difference of 25% in rifampin AUC_{0-24}. However, with a number of 25 individuals included in the group with a normal BMI, it can be calculated that the present study could still detect a 24% difference in the AUC_{0-24} of total rifampin with a significance level of 0.05 and a power of 80%. As a second limitation, it can be argued that the BMI of the well-nourished group was considerably low (median, 19.6 kg/m^2; range, 18.7 to 22.6), resulting in a relatively small difference from the malnourished group (16.3 kg/m^2; 16.1 to 16.4). However, also when comparing the severely malnourished subgroup with well-nourished patients and when analyzing BMI as a continuous variable, nutritional status and BMI do not appear to have a major effect on total and protein-unbound pharmacokinetic parameters of rifampin. Third, it should be considered that all participants were Indonesian, and it cannot be excluded that the effect of malnutrition on rifampin pharmacokinetics is different in people with another racial or genetic background. A final limitation is that we did not include pharmacogenetic analyses. Pharmacogenetic polymorphisms are proposed to be an important factor in the high variability in rifampin exposure. More specifically, polymorphisms of the SLCO1B1 gene have been associated with lower rifampin exposure (16, 34, 42, 43). To our knowledge, the potential impact of such polymorphisms in Indonesian TB patients has not been investigated. According to Niemi et al., the c.463C<A polymorphism, which has been associated with decreased rifampin exposures, is present in only 0 to 3% of the East Asian population (44). This indicates that at least this specific polymorphism is of minor importance in our study population and will not affect differences in rifampin exposures between malnourished and well-nourished patients.

To summarize, severely malnourished, malnourished, and well-nourished Indonesian TB patients showed no clear difference in total and protein-unbound pharmacokinetic parameters of rifampin during the intensive phase of TB treatment. Similarly, BMI and rifampin (total and unbound) pharmacokinetic parameters did not show any significant correlation. Significantly higher plasma concentrations (total and unbound) were found in females, also when corrected for BMI. Finally, a marked 2-fold interindividual variation in the unbound rifampin fraction was observed. This large interindividual variability in the free fraction of rifampin shows that measurement of solely total concentrations could be misrepresentative of the actual exposure, suggesting that it should be considered to evaluate protein-unbound rifampin concentrations along with total plasma concentrations when studying exposure-response relationships for this pivotal TB drug.

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